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Recurrent phenotypic selection and recurrent selection based on combining ability in tetraploid bahiagrass

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Abstract

Paspalum notatum Flüggé is an apomictic grass used for forage and turf. The recent generation of a sexual synthetic tetraploid population of the species provides the unique advantage to exploit heterosis by accumulating favorable alleles through recurrent selection cycles. The objective was to evaluate recurrent phenotypic selection (RPS) and recurrent selection based on combining ability (RSCA) as breeding methods. The best sexual genotypes in terms of warm-season growth from a sexual synthetic tetraploid population were selected by RPS and RSCA. The selected genotypes were polycrossed and two new sexual populations were created, which were crossed with superior apomictic genotypes. Both methods allowed obtaining families that were evaluated for summer, fall, and spring growth. Breeding values and heterosis were also calculated. Summer growth for RPS families was greater than for RSCA families (33.1 and 29.08 g plant^{-1}, respectively); however, fall, spring, and accumulated growth were similar. The breeding value for RPS sexual parents was greater than for RSCA sexual parents for summer growth, whereas those for fall and spring growth were similar. A greater level of families with heterosis (80%) was observed for RPS than for RSCA (30%) for summer growth, whereas the levels for fall and spring growth were similar. These results indicate that both breeding methods were successful in improving tetraploid *P. notatum*. They increased the mean value of the progenies and produced families superior for forage yield by accumulating favorable alleles.

1 | INTRODUCTION

Many apomictic warm-season grasses are cultivated around the world, and they play an important role in the economy of beef cattle production systems of the tropics and subtropics (Moser, Burson, & Sollenberger, 2004). For example, in Brazil, which is one of the world's largest beef producer and exporter countries, most of the beef production is based on cultivated apomictic forages (Jank, Barrios, do Valle, Simeão, & Alves, 2014). Within the apomictic warm-season grasses, the genera with greatest importance are *Brachiaria*, *Paspalum*, *Panicum*, and *Cenchrus* (Blount & Acuña, 2009; Jank et al., 2014; Moser et al., 2004).

Apomixis, which is a type of asexual reproduction by seeds, offers a unique opportunity for developing and using superior hybrid genotypes (Miles, 2007). Apomictic individuals showing superior characteristics will be fixed without loss of vigor

Abbreviations: ISSR, inter-simple sequence repeat; PCR, polymerase chain reaction; RPS, recurrent phenotypic selection; RSCA, recurrent selection based on combining ability; SSTP, sexual synthetic tetraploid population.

or change in genotype for an unlimited number of generations (Hanna, 1995; Hanna & Bashaw, 1987).

Genetic improvement of apomictic species has been achieved mostly through selection of ecotypes that showed desirable characteristics (Vogel & Burson, 2004). This breeding method has been very successful and has allowed the release of many cultivars (Blount & Acuña, 2009). However, it is limited by the difficulty of finding ecotypes that combine traits of interests (Miles, 2007).

Improving apomictic grasses by hybridization started when sexual plants with the same ploidy level as apomictic plants were discovered or created by doubling the chromosomes of naturally occurring sexual diploid genotypes (Bashaw, 1962; Burton & Forbes, 1960; Pinheiro, Pozzobon, do Valle, Penteado, & Carneiro, 2000; Sartor, Quarin, & Espinoza, 2009; Savidan, 1975; Swenne, Louant, & Dujardin, 1981). In addition, hybridization allowed studies of the inheritance of apospory, which determined that in forage grasses, this trait is controlled by a single dominant Mendelian factor (Ozias-Akins & van Dijk, 2007). Several breeding techniques were proposed to generate highly apomictic hybrids with superior characteristics that could be released as new cultivars (Acuña, Blount, Quesenberry, Kenworthy, & Hanna, 2011; Gobbe, Longly, & Louant, 1983; Hanna, 1995; Pernès, Réné-Chaume, Réné, & Savidan, 1975; Savidan, 2000). Most of these breeding schemes highlighted the advantages of apomixis in the utilization of hybrid vigor or heterosis; however, none of these schemes was developed for accumulating and exploiting heterotic effects (Miles, 2007).

Bahiagrass (*Paspalum notatum* Flüggé) is a valuable apomictic forage species in beef cattle production systems in the tropics and subtropics. This species is characterized by its wide distribution and dominance in the grasslands of South America (Ortiz et al., 2013), as well as by its cultivation in several countries around the world (Blount & Acuña, 2009). It has an apomictic tetraploid cytotype that dominates most of the area where the species is distributed, and a sexual diploid, which has been found naturally located in northeastern Argentina (Gates, Quarin, & Pedreira, 2004). Generation of sexual tetraploid plants by doubling the chromosomes of diploid plants (Burton & Forbes, 1960; Quarin, Espinoza, Martínez, Pessino, & Bovo, 2001, 2003; Quesenberry, Dampier, Lee, Smith, & Acuña, 2010), elucidating details of apospory inheritance (Martínez, Urbani, Quarin, & Ortiz, 2001), and the identification of molecular markers tightly linked to apospory (Martínez, Hopp, Stein, Ortiz, & Quarin, 2003; Stein et al., 2007) have allowed developing breeding programs for the species at the tetraploid level (Zilli et al., 2018). These programs are based on the generation of hybrid progenies from crosses between experimental sexual tetraploid genotypes and apomictic ecotypes (Blount & Acuña, 2009). New hybrids with superior characteristics were obtained from these crosses, and ~10% of these hybrids inherited the apomixis trait with a high level of expression that allowed fixing the superior characteristics (Acuña et al., 2011; Zilli et al., 2015). However, one of the limitations for the genetic improvement in this species through hybridizations is the low diversity present in the available sexual tetraploid germplasm. In response, a sexual synthetic tetraploid population (SSTP) has been recently generated following a four-step procedure (Zilli et al., 2018). First, F_1 hybrid progenies were obtained by crossing a few experimental sexual genotypes and a group of apomictic genotypes collected in different places of the Americas, with the objective of transferring the diversity of apomictic germplasm to sexual genotypes. Then, F_1 hybrids were classified by their mode of reproduction as sexual or apomictic. The most diverse sexual hybrids were selected and polycrossed, and a sexual synthetic population characterized by its wide diversity was obtained (Zilli et al., 2019). Since sexuality behaves as a recessive trait in bahiagrass (Martínez et al., 2001), it is possible to maintain this mode of reproduction in a sexual synthetic population over breeding cycles (Zilli et al., 2018). The diversity present in the apomictic ecotypes was successfully transferred to the SSTP, as demonstrated using molecular and morphological markers by Zilli et al. (2019).

Considering the importance of heterosis for breeding apomictic species and the availability of a highly diverse sexual synthetic tetraploid population of *P. notatum* (Zilli et al., 2019), new breeding techniques could be developed to improve the tetraploid germplasm of bahiagrass. Cycles of selection and crosses between the sexual synthetic population and apomictic genotypes could be implemented and thus obtain progenies with superior characteristics. One alternative was the use of recurrent phenotypic selection (RPS), as Burton (1974) did in the sexual diploid germplasm of the species. After four selection cycles, an increase in forage yield of 18% was achieved by using RPS in diploid bahiagrass. Another method that may be used is recurrent selection based on combining ability (RSCA), as proposed to obtain improved apomictic hybrids of *Brachiaria* (Miles, 2007; Worthington & Miles, 2015). This technique, widely used in sexually reproducing maize (*Zea mays* L.) (Fehr, 1991; Hallauer, Carena, & Filho, 2010), involves crossing individuals of the sexual tetraploid population by a common tester (superior apomictic genotype or genotypes), evaluating the progeny to determine the combining ability of the individuals of the sexual population, and polycrossing the selected sexual individuals by combining ability to develop an improved population. Selection on general or specific combining ability (Comstock, Robinson, & Harvey, 1949; Hull, 1945; Jenkins, 1940) in each cycle allows the accumulation of favorable alleles that contribute to heterosis. It is expected that progenies obtained by crossing sexual genotypes selected on combining ability with apomicts would exhibit a greater level of heterosis, due to the accumulation of genes contributing to heterotic effects.

Taking into account the information reported above, it would be of great interest to evaluate the use of recurrent selection in improving apomictic species, using *P. notatum* as a model for apomictic forages. This would be possible by evaluating in parallel the efficiency of the use of RPS and RSCA starting with the synthetic sexual tetraploid population. The objectives of this research were (i) to develop two sexual tetraploid populations of *P. notatum*, one by RPS and another by RSCA, (ii) to generate hybrids between the sexual tetraploid genotypes obtained by RPS and RSCA, and a group of apomictic tetraploid genotypes, (iii) to evaluate the performance of the progenies obtained by the two selection methods, and (iv) to determine the occurrence of heterosis for forage yield.

2 | MATERIALS AND METHODS

2.1 | Plant material

A group of 308 plants from the SSTP of *Paspalum notatum*, recently developed by Zilli et al. (2018), was used for this research. These plants were transplanted to the field in 1- by 1-m, single-plant experimental units. Spring–summer growth was evaluated in October and November 2014, with the objective of selecting 30 individuals. In October, spring growth was estimated using a qualitative, 1–5, visual scale (1 = least growth, 5 = greatest growth). This evaluation allowed preselection of 70 individuals that exhibited the greatest spring growth. Then, all 308 plants were cut at 5-cm stubble height using a sickle bar mower to assess 30-d regrowth. In November, biomass production of the preselected plants was evaluated. Forage of individual plants was harvested at 5-cm stubble height, the fresh weight of harvested material was recorded, and the material was dried at 60°C for 48 h. Dry material was weighed to determine the amount of harvested biomass. Based on these evaluations, the 30 plants that showed greatest spring–summer growth were selected.

2.2 | Recurrent selection based on combining ability

The 30 plants of the SSTP selected by their greater spring–summer growth were used in a breeding scheme called RSCA, as described by Miles (2007) and which was modified in this work for its use in *P. notatum*. This breeding technique includes four stages (Figure 1a). In Stage 1, the 30 plants of the SSTP with greatest spring–summer growth were crossed by a group of five elite apomictic genotypes (Table 1), which were used as wide genetic base testers. Crosses were made in January 2015 under controlled conditions during anthesis (around 6:30 AM) as described by Acuña, Blount, Quesenberry, Kenworthy, and Hanna (2009). A day before anthesis, rooted culms of each sexual parent bearing spikelets were placed in a 1-L container with water in a greenhouse, and inflorescences were covered with a glassine bag. The same procedure was used with the apomictic parents, but they were placed a day before anthesis in a laboratory near an open window. Pollen of each apomictic parent was collected in similar proportions in a glassine bag. Inflorescences of sexual plants were dusted with the bulk pollen. This procedure was repeated over 3–4 d until all spikelets of the female parent finished flowering. Pollinated inflorescences were bagged to avoid pollen contamination from undesired sources. The containers were then placed in a shaded and fresh part of the greenhouse where they remained for 20 d after pollination. Inflorescences from the sexual parents were then manually threshed and seeds were separated using a seed blower (Seedburo Equipment Company 1022W).

Hybrid progeny, 24 individuals per each of 30 families, obtained during Stage 1 were transplanted to a field located near the city of Corrientes on November 2015, in 1- by 1-m single-plant experimental units, in a randomized complete block designed with three replications. Summer growth of these progenies was evaluated in February 2016. First, the plants were evaluated on the same 1–5 visual scale described above. Second, forage of individual plants was cut using a sickle bar mower at 5-cm stubble height, the fresh weight of the harvested material was recorded, and the material was dried at 60°C for 48 h and then weighed. Based on this evaluation, 10 parental sexual genotypes whose progenies showed the greatest summer growth were selected (Stage 2). In February 2016, the 10 genotypes selected in Stage 2 were polycrossed using the methodology described by Burton (1974) (Stage 3). One day before anthesis, rooted culms of the selected genotypes bearing inflorescences were collected and immediately placed in a 1-L container with water. Two inflorescences per genotype, each appropriately labeled, were enclosed inside of the same glassine bag to facilitate cross-pollination and shaken for 3–4 d at anthesis. Inflorescences of each genotype were then enclosed separately and placed in a shaded corner of the glasshouse, where they remained for 30 d after pollination. Then, 10 genotypes, Cycle 1 of RSCA (RSCA-C1), that resulted from the intercross were taken at random at seedling stage and crossed to the same five apomictic tester genotypes (Stage 4). Crosses were made as was described in Stage 1. These crosses gave rise to 10 families each represented by 32 randomly selected individuals. In November 2017, these families and their sexual and apomictic parents were transplanted to the field in 1- by 1-m, single-plant experimental units, near the city of Corrientes, Argentina, in a randomized complete block design with four replications.

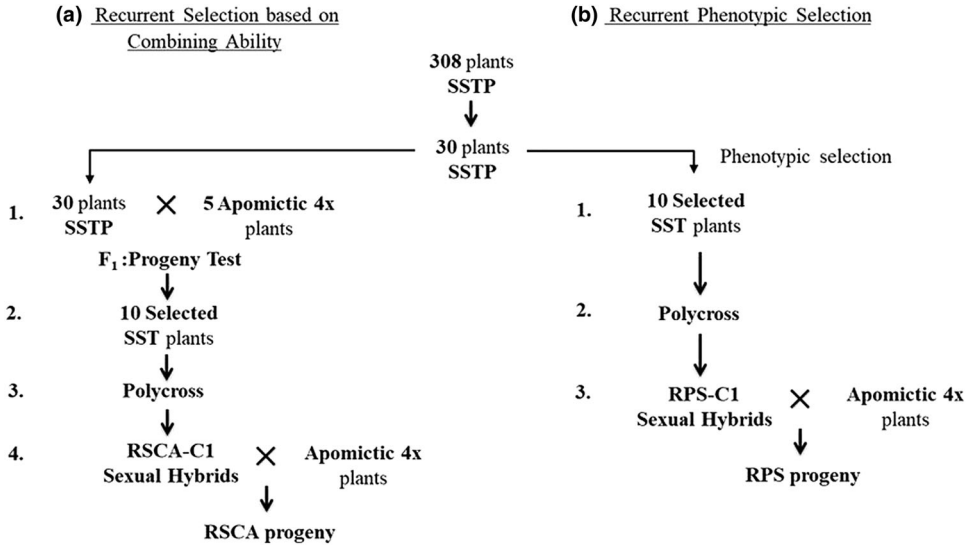

FIGURE 1 (a) Scheme of recurrent selection based on combining ability applied to the improvement of a sexual synthetic tetraploid population (SSTP) of *P. notatum*. (b.) Scheme of recurrent phenotypic selection applied to the improvement of SSTP of *P. notatum*. Numbers indicate stages of each breeding method. SST, sexual synthetic tetraploid; RSCA, recurrent selection based on combining ability; RPS, recurrent phenotypic selection; C1, Cycle 1

TABLE 1 Identification and origin of elite apomictic genotypes of *Paspalum notatum* used as testers for a selection procedure based on combining ability

Accession	Origin
Boyero UNNE	Hybrid cultivar obtained by crossing a sexual tetraploid genotype (SWSB) and an apomictic tetraploid genotype (Q4117). Registered in the Instituto Nacional de Semillas in the year 2012 (Reg. no. 3213) as the first hybrid cultivar of *P. notatum* (Urbani et al., 2017).
UF3	Hybrid obtained by crossing a sexual tetraploid genotype (Q4205) (Quarin et al., 2003) and an apomictic tetraploid genotype (Tifton 7). Introduced to Argentina through an agreement between the Universidad Nacional del Nordeste and the University of Florida.
UF13	Hybrid obtained by crossing a sexual tetraploid genotype (106) (Acuña, Blount, Quesenberry, Hanna, & Kenworthy, 2007) and an apomictic tetraploid genotype (Tifton 7). Introduced to Argentina through an agreement between the Universidad Nacional del Nordeste and the University of Florida.
UF93	Hybrid obtained by crossing a sexual tetraploid genotype (106) (Acuña et al., 2007) and an apomictic tetraploid genotype (Argentine). Introduced to Argentina through an agreement between the Universidad Nacional del Nordeste and the University of Florida.
UF122	Hybrid obtained by crossing a sexual tetraploid genotype (Q4188) (Quarin et al., 2003) and an apomictic tetraploid genotype (Argentine). Introduced to Argentina through an agreement between the Universidad Nacional del Nordeste and the University of Florida.

2.3 | Recurrent phenotypic selection

The same 30 sexual plants from the SSTP used in the RSCA scheme were used in another breeding method called RPS developed by Burton (1974), with some modifications. The breeding technique used in this research involved three stages (Figure 1b). In Stage 1, from the same 30 sexual genotypes of the SSTP, 10 were selected on their superior spring growth. For this, spring growth was first estimated using the 1–5 visual scale described above. Then, biomass production of each individual plant was evaluated in November 2014 as described above. Based on these two estimations of spring growth, selections were made in Stage 1 (Figure 1b). In February 2015, the 10 selected genotypes were polycrossed (Stage 2) to generate a Cycle 1 of RPS (RPS-C1), as was described in Stage 3 of the RSCA scheme. In Stage 3, 10 genotypes from RPS-C1 were taken at random at the seedling stage and crossed to the same set of five apomictic genotypes used in Stage 3 of the RSCA scheme. Crosses were made as described above. These crosses gave rise to 10 families, each represented by 32 randomly selected individuals. In November 2017, these families and their parents were transplanted into the field, near

the city of Corrientes, Argentina, in 1- by 1-m single-plant experimental units, in a randomized complete block design with four replications.

2.4 | Efficiency of the crossing method and paternity test

The efficiency of the crossing technique was evaluated using fingerprints based on inter-simple sequence repeat (ISSR) molecular markers in both selection procedures: RSCA (Stages 1 and 4) and RPS (Stage 3). The analysis was performed in a sample of the generated progeny (16% of total plants).

Genomic DNA was extracted from young leave of each individual using the protocol described by Brugnoli et al. (2014). The integrity and concentration of genomic DNA were estimated using known standards and separated by electrophoresis in 1% w/v agarose gel in 1 × TAE (40 mM Tris-HCl, 5 mM sodium acetate, 0.77 mM EDTA, pH 8.0) at 40 V for 1 h. Gels were stained with ethidium bromide (1 μg ml^{-1}) for 30 min, and DNA samples were visualized under ultraviolet light and photographed with a GelDoc-It imaging system (UVP). For their use in polymerase chain reaction (PCR) amplifications, DNA samples were adjusted to 10 ng μl^{-1}.

The ISSR primers were used for PCR amplifications according to the methodology described by Zilli et al. (2015) under the same conditions. Fragments PCR were separated by electrophoresis in 2% w/v agarose gel in 1 × TAE (40 mM Tris-HCl, 5 mM sodium acetate, 0.77 mM EDTA, pH 8.0) at 70 V for 3 h. Gels were stained with ethidium bromide (1 μg ml^{-1}) and fragments were visualized under ultraviolet light, photographed, and stored for further analysis with a GelDoc-It imaging system (UVP).

Those progenies that amplified at least two specific markers from the same apomictic male parent were considered hybrids. However, those progenies that did not amplified any specific marker of the apomictic parent were considered products of self-pollination of the sexual parent (Supplemental Figure S1a).

In addition, a paternity test was conducted to determine whether an apomictic plant was the male parent of a particular hybrid. This analysis was performed by detecting at least two male-parent-specific molecular markers for each hybrid (Supplemental Figure S1b).

2.5 | Comparative evaluations

The 20 progenies obtained by crossing RSCA-C1 and RPS-C1 genotypes with the group of five apomictic tester genotypes were evaluated, together with female and male parents, in the field for summer, fall, and spring growth. These 20 progenies and their parents were transplanted into the field, near the city of Corrientes, Argentina, in 1- by 1-m single-plant experimental units, in a randomized complete block design with four replications. Each progeny was represented by 32 randomly selected genotypes.

In March, May, and November 2018, forage of individual plants was harvested by hand at 5-cm stubble height, the fresh material was weighed and dried at 60°C for 48 h, and dried material was weighed. Performance of RSCA and RPS progenies were compared by an ANOVA and mean comparisons using the LSD test. In addition, the breeding value of the sexual parent was calculated as the difference between its mean value and the mean value of its progeny. Statistical significance of those differences was evaluated by the student's *t* test. These analyses were performed using the Info-Stat software (Di Rienzo et al., 2017).

2.6 | Relationship between paternity and forage yield

A linear regression test between percentage paternity of the male parent that contributed most to each family and family forage yield was conducted to determine if seasonal forage yield from RSCA-C1 and RPS-C1 progenies were influenced by contribution of particular male parents. These analyses were performed using the Info-Stat software (Di Rienzo et al., 2017).

2.7 | Heterosis analysis

The occurrence of heterosis in the progenies obtained by the two breeding techniques was evaluated following the methodology proposed by Marcón et al. (2019). This methodology allowed determination of the genetic effects associated with the expression of forage yield and the proportion of families exhibiting heterosis. Genetic effects are represented by the additive gene effects, which are associated with the gene or allele values, and by the nonadditive gene effects that are associated to the genetic interaction between alleles of the same gene (dominance) or alleles of different genes (epistasis). The methodology proposed that the additive and dominance effects are expressed by the parents with greater (P1) or lesser (P2) phenotypic expression of the character under study, and the hybrid family (F). In this way, the positive complete dominance effect (CD+) is represented by the parent with greater phenotypic expression of the character, the negative complete dominance effect (CD−) is represented by the parent with lower phenotypic expression, and the additive genetic effect (Ad) is given to the mean value of both parents (MPV). Genetic effects involved into the expression of traits under study were evaluated using a student's *t* test. The

mean value of each family was compared with the MPV, P1, and P2. Genetic effects considered in this study were: negative overdominance (OD−), negative complete dominance (CD−), negative partial dominance (PD−), additivity (Ad), positive partial dominance (PD+), positive complete dominance (CD+), and positive overdominance (OD+).

Once the genetic effects of the hybrids were determined, the proportion of families with heterosis were calculated. Those families that exhibited positive partial dominance, positive complete dominance, and positive overdominance were considered as superior families.

3 | RESULTS

3.1 | Efficiency of the crossing method

The efficiency of the crossing technique used in Stage 1 of RSCA scheme was evaluated in 116 random individuals (four individuals per each of 29 families) using eight ISSR primers. Nine individuals (8%) did not amplify any specific marker from the male parent and were considered products of self-pollination. Thus, the efficiency of the crossing method was 92% (Table 2). Moreover, this technique allowed identification of the apomictic parent Boyero UNNE as the male parent of 31% of hybrids, UF13 as the male parent of 30% of hybrids, and UF3 and UF93 as male parents of 11 and 1% of hybrids, respectively (Table 3). In the remaining hybrids, this estimation could not be made since they amplified at least two molecular markers that were present in more than one male parent.

In addition, the efficiency of the crossing technique performed in Stage 4 of the RSCA scheme and Stage 3 of the RPS scheme was evaluated. In the case of RSCA, 10 ISSR primers were performed on 40 random individuals (four individuals per family). Only two individuals (5%) did not amplify any specific marker from the male parent and were considered as product of self-pollinization of the sexual parent. Hybridization efficiency was 95% (Table 2). Boyero UNNE and UF13 were male parents of 15 and 17.5% of hybrids, respectively. UF3, UF93, and UF122 were identified as male parent of 10, 7.5, and 5% of hybrids, respectively (Table 3). This estimation could not be performed in the rest of the hybrids, since they amplified at least two molecular markers that were present in more than one male parent. In the case of RPS, six ISSR primers were performed in 40 random individuals (four individuals per family). Three individuals did not amplify any specific marker of the male parent and were classified as products of self-pollination. Hybridization efficiency was 92.5% (Table 2). Regarding the percentage of paternity of each apomictic parent, it was observed that UF93 and UF122 were male parents of 15 and 20% of hybrids, whereas UF3 and UF13 were male parents of 2.5 and 10% of hybrids, respectively (Table 3). This estimation could not be made on the rest of the hybrids, since they amplified at least two molecular markers that were present in more than one male parent.

3.2 | Performance of the progeny and the relationship with paternity

Performance of the progenies obtained by both breeding methods was evaluated for summer, fall, spring, and accumulated growth. In summer, significant differences were observed between RPS and RSCA progenies ($p < .01$). Mean forage yield was 33.1 and 29.08 g plant^{-1}, respectively (Table 4, Supplemental Table S1). Regarding fall, spring, and accumulated forage yield, significant differences were not observed between RPS and RSCA progenies ($p > .05$) (Table 4, Supplemental Table S1). No significant linear regression was observed between paternity percentage and summer, fall, and spring forage yield of RSCA-C1 and RPS-C1 progenies.

3.3 | Breeding value

A positive breeding value of the sexual parent was defined as the change in the mean value of RSCA and RPS progenies, produced by the effect of the female. This value was expressed as the proportion of families with a positive increase in their mean value in comparison with the mean value of the female parent. The frequency of families that showed different amount of increase in forage production—that is, a positive breeding value—is observed in Figure 2. For summer growth, significant differences in breeding values were observed between RSCA and RPS ($p = .04$). For RSCA, 30% of families were within 1–40%, whereas 10% of families were in the intermediate interval (61–80%). In the case of RPS, it was observed that 70% of the families fell between 61 and >100% of breeding value, whereas 10% of families were in the lowest interval (Figure 2). Concerning fall and spring growth, significant differences were not observed between RSCA and RPS breeding values ($p > .05$).

3.4 | Genetic effects

Genetic effects involved in the expression of summer, fall, and spring growth traits were evaluated in two populations of *P. notatum*, one of them generated by RSCA and the other one by RPS, to determine the proportion of families with superior characteristics. For summer forage yield, the predominant genetic effects in RSCA progenies was additivity (Ad.), which was present in 30% of the families, whereas in RPS progeny, the positive dominance (D+)

TABLE 2 Number of molecular markers (MM), number of individuals analyzed, number of individuals that amplified and that did not amplify specific molecular markers from the male parent, and hybridization efficiency of the crossing technique used in the recurrent selection based on combining ability (RSCA) and recurrent phenotypic selection (RPS) schemes

Method	No. MM	No. individuals analyzed	No. individuals		Hybridization efficiency
			Amplified MM	Not Amplified MM	
					%
RSCA					
Stage 1	8	116	107	9	92.0
Stage 4	10	40	38	2	95.0
RPS					
Stage 3	6	40	37	3	92.5

TABLE 3 Percentage paternity of each apomictic genotype used as male parent in crosses with sexual genotypes of *P. notatum* improved by recurrent selection based on combining ability (RSCA) and recurrent phenotypic selection (RPS)

Breeding method	Paternity					
	UF3	UF13	UF93	UF122	Boyero UNNE	Not determined[a]
	%					
RSCA	10	17.5	7.5	5	15	37.5
RPS	2.5	10	15	20	0	42.5

[a]"Not determined" represents a group of hybrids that amplified at least two molecular markers that were present in more than one male parent.

TABLE 4 Summer, fall, and spring growth, represented as forage yield, of the recurrent selection based on combining ability (RSCA) and recurrent phenotypic selection (RPS) progenies

Method	Forage yield							
	Summer progeny		Fall progeny		Spring progeny		Accumulated progeny	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	g plant^{-1}							
RSCA	29.08	1.02	13.52	0.45	62.72	3.44	105.30	4.64
RPS	33.10	1.11	12.99	0.38	56.76	3.29	102.74	4.64
p value	**		ns[a]		ns		ns	

**Significant at the .01 probability level.

[a]ns, not significant.

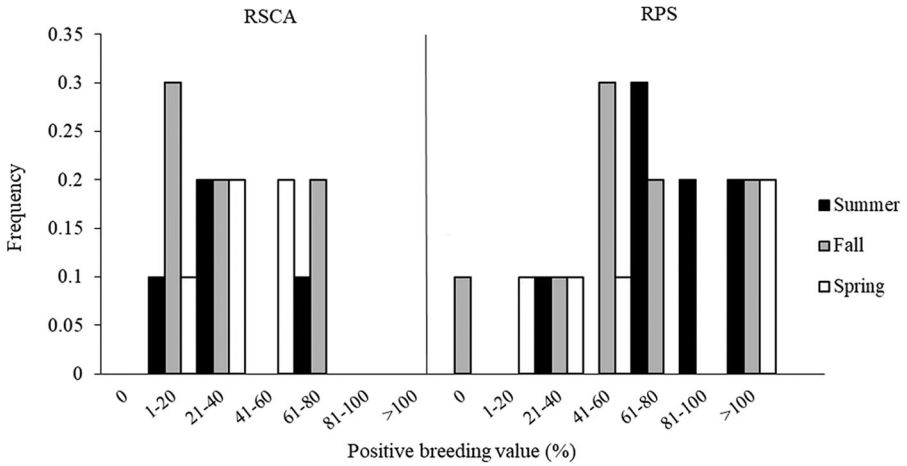

FIGURE 2 Proportion of *P. notatum* families obtained by recurrent selection based on combining ability (RSCA) and recurrent phenotypic selection (RPS) with positive increase in their mean value regarding the sexual parent mean value for summer, fall, and spring growth

TABLE 5 Genetic effects related to the expression of summer, fall, and spring growth, represented as the percentage of families of two populations of *P. notatum* obtained by recurrent selection based on combining ability (RSCA) and recurrent phenotypic selection (RPS)

	Families							
	Genetic effects[a]							
Traits	OD(−)	D(−)	PD(−)	Ad.	PD(+)	D(+)	OD(+)	Und.
	%							
Summer growth								
RSCA	20	20	0	30	0	20	10	0
RPS	20	0	0	0	0	60	20	0
Fall growth								
RSCA	0	20	0	40	0	40	0	0
RPS	0	10	0	50	0	30	10	0
Spring growth								
RSCA	30	30	0	0	0	10	0	30
RPS	20	20	0	0	0	20	0	40

[a]OD(−), negative overdominance; D(−), negative dominance; PD(−), negative partial dominance; Ad., additivity; PD(+), positive partial dominance; D(+), positive dominance; OD(+), positive overdominance; Und., undefined.

genetic effect predominated (60% of the families) (Table 5, Supplemental Table S2). Regarding fall growth, the genetic effects that predominated in the RSCA progeny were the additivity and the positive dominance, each present in 40% of the families. However, in RPS progenies, the predominant genetic effect was the additivity, which was present in 50% of the families (Table 5, Supplemental Table S2). In the case of spring growth, the genetic effects that predominated in RSCA and RPS progeny were the negative dominance and overdominance, which were present in 60 and 40% of the families, respectively (Table 5, Supplemental Table S2).

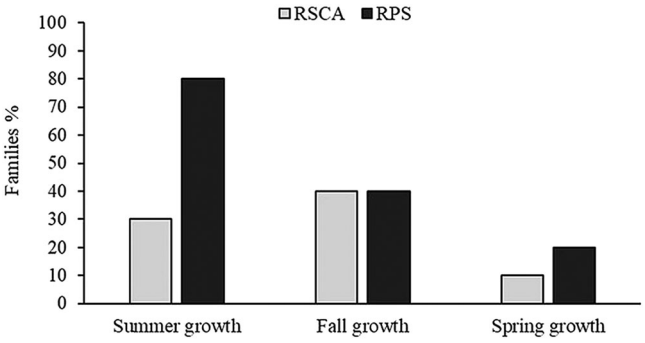

FIGURE 3 Percentage of families that display heterosis in two populations of *P. notatum*, one of them obtained by recurrent selection based on combining ability (RSCA) and the other one by recurrent phenotypic selection (RPS)

3.5 | Occurrence of heterosis

The proportion of families that exhibited heterosis differs between the breeding methods, and between the growing seasons. For summer growth, the proportion of superior families was greater for RPS than for RSCA; however, for fall and spring growth, the progenies obtained by both techniques showed similar proportions of superior families (Figure 3). The proportion of families, obtained by RSCA, that displayed heterosis for summer, fall, and spring growth were 30, 40, and 10%, respectively, whereas the proportion of families obtained by RPS that showed heterosis for summer, fall, and spring growth were 80, 40, and 20%, respectively (Figure 3).

4 | DISCUSSION

The availability of a sexual synthetic tetraploid bahiagrass population creates new opportunities to improve the species by accumulating favorable alleles by recurrent selection cycles. In this work, the use of RPS and RSCA to improve the sexual tetraploid germplasm of *P. notatum* was evaluated. In addition, crosses between the improved sexual hybrids and apomictic genotypes were assessed to exploit the benefits of apomictic reproduction.

Recurrent phenotypic selection has been effective in major crops such as maize, where it increased grain oil content 2.6 times after five selection cycles (Sprague & Brimhall, 1950), or grain yield, where a 38% increase was obtained after 13 selection cycles (Gardner, 1961). In forage species, this method has been also effective to improve forage yield and resistance to diseases and insects (Bruckner, Raymer, & Burton, 1991; Burton, 1974; Hill, Hanson, & Busbice, 1969; Miles, Cardona, & Sotelo, 2006). Burton (1974) reported that the use of recurrent restricted phenotypic selection (RRPS), a modified form of RPS, was successful in improving forage yield of diploid bahiagrass. Four selection cycles increased forage yield 18% over 'Pensacola'. In this work, as in Burton (1974), the RPS method was efficient at the tetraploid level,

since after one selection cycle, ∼50% of families showed an increase from 41 to 100% in forage yield with respect to the sexual parent. This greater increase in forage yield than in Burton (1974) may be related to the selection intensity used, which in our work was 3%, whereas in Burton (1974) it was 20%. This difference may be associated with the fact that we used a highly diverse sexual base population (Zilli et al., 2019), and also with environmental differences between experiments. In addition, this method was effective to improve *Medicago sativa* L. (lucerne), a forage legume (Dudley, Hill, & Hanson, 1963). The authors released a new lucerne cultivar resistant to rust (*Uromyces striatus* Schroet. var. *medicaginis*) and to lucerne leafhopper (*Empoasca phabae* Harris) after seven selection cycles, and with a selection intensity of 5%. The new cultivar also yielded 10% more forage than other cultivars. The greatest resistance was obtained during the first selection cycle, since they started with two highly diverse base populations and applied a high selection intensity, as was done in our study. This may explain the increase in the progeny mean value with respect to the mean value of female parent obtained in this work during the first selection cycle.

Recurrent selection based on combining ability is a breeding method with many variants depending on the tester used. In maize, it was reported that all of them were effective to improve grain yield (Hallauer & Darrah, 1985; Hallauer et al., 2010; Horner, Lundy, Lutrick, & Chapman, 1973). Weyhrich, Lamkey, and Hallauer (1998) evaluated different variants to this method and observed that after four selection cycles and with a selection intensity of 20%, yield increased between 4.5 and 0.6% per cycle. They reported that one of the most effective methods was recurrent selection based on half-sib progeny test, which is similar to that used in this study. In our work, this method was also effective, since after one selection cycle, a progress in forage yield between 21 and 40% was achieved in 20% of families in comparison with the sexual parent, and in another 20% of families achieved a gain of from 61–80%. The greater progress obtained in this work in one selection cycle than obtained by Weyhrich et al. (1998) may be related to the tester and the selection intensity used. Weyhrich et al. (1998) used a unique improved genotype as a tester, and in this study, a group of improved genotypes were used with the objective to select for improved general combining ability. In addition, the selection intensity applied in our study was greater than in Weyhrich et al. (1998), so response to selection was quickly exhibited. In lucerne, a recurrent selection method based on progeny test allowed Riday and Brummer (2005) to identify those individuals of subspecies *falcata* that combined better with individuals from subspecies *sativa*, used as a tester. In addition, they reported that individuals selected by this method exhibited a greater level of heterosis than those individual not selected. In our study, this method was also effective, since it allowed identifying those individuals that produced progenies with greater heterosis.

In apomictic species, the efficiency of RSCA has not been proven until now. In addition, Miles (2007) proposed a breeding scheme for brachiariagrass [*Brachiaria* (Trin.) Griseb.] that we took as model. In our work, a group of apomictic genotypes were used as testers, instead of a single outstanding cultivar as proposed by Miles (2007). This was due to the fact that we were interested in identifying those genotypes that combined well with a group of testers (i.e., the ones that had greater general combining ability).

Comparing the performance of RPS and RSCA progenies, both breeding methods were effective to improve tetraploid *P. notatum*, since progenies with heterosis were obtained. Nonetheless, RPS progenies yielded 14% more forage and showed a greater breeding value and heterosis for summer yield than progenies generated by RSCA. For fall and spring forage yield, both progenies were similar, as was expected since selection of the best sexual parents was on summer yield. The results reported in this work differ from those reported for other species, where progeny test selection methods were more effective than RPS. For example, in maize, Weyhrich et al. (1998) showed that selection based on half-sib or full-sib progeny tests were more efficient than recurrent phenotypic selection, since they allowed gain in hybrid grain yield between 1.4 and 1.6% per cycle in comparison with 0.6% for RPS. Another example is the work in lucerne reported by Riday and Brummer (2005), where parental selection based on half-sib progeny test increased forage yield two to nine times more than recurrent phenotypic selection. On the other hand, Hill and Leath (1979) reported in lucerne that half-sib and full-sib progeny test selection did not differ from recurrent phenotypic selection for resistance to a leaf disease produced by *Leptosphaerulina briosiana* (Pollacci) J. H. Graham & Luttr. These results are similar to those reported in this study. Hill and Leath (1979) proposed that it could be related to the low selection intensity used and the low diversity of the base population.

Analyzing the paternity test, it was observed that in RSCA and RPS progeny, all apomictic male parents contributed, but in different proportions, which may account for the differences observed. However, no significant relationship was observed between the percentage paternity and forage yield.

In this work, the similar results obtained by RPS and RSCA may be related to the highly diverse base sexual population used (Zilli et al., 2019), and the high selection intensity employed. This combination of causes resulted in a high response to selection in the first selection cycle, as Dudley et al. (1963) reported. Another reason may be related to the efficiency of the polycross technique used, which was developed by Burton (1974). Another potential reason may relate to the fact that 50% of the selected sexual plants were the same for both methods. However, this last hypothesis is limited by

the large contribution of the other 50% of the selected sexual genotypes in each polycross.

Regarding the occurrence of heterosis in *P. notatum*, we have already demonstrated that crossing very distant parents favors increasing the level of heterosis for forage yield (Marcón et al., 2019). In this work, given that the SSTP and apomictic genotypes used as male parents have different origins, a high genetic distance could be expected between them. This may explain the similarity in the proportion of heterotic families between RPS and RSCA. However, to confirm this hypothesis, it would be necessary to determine the genetic distance between sexual and apomictic genotypes by using molecular markers, as was done in Marcón et al. (2019). Although the greatest selection response is expected after the first selection cycle, it would be of great interest to evaluate one or two more selection cycles to then compare the progress obtained in each of them.

Analyzing the genetic effects involved in the occurrence of heterosis of both progenies, it was observed that complete dominance was predominant. This may indicate that both selection methods allowed accumulation of favorable alleles with partial or complete dominance in one selection cycle. These results are similar to those reported before in *P. notatum* (Marcón et al., 2019) and in *Brachiaria* (Matias et al., 2018), where heterosis was mainly due to dominance genetic effects, which may be supporting the hypothesis that in apomictic species, hybrid vigor is due to the accumulation of favorable, dominant alleles rather than to interallelic interactions.

In conclusion, the use of both recurrent selection methods allowed obtaining two sexual tetraploid populations of *P. notatum* starting from a SSTP previously generated. It was possible to generate hybrids between the sexual genotypes selected by RPS and RSCA to be used as female parents, and a group of apomictic genotypes used as male parents. In addition, the crossing technique used was highly effective in obtaining hybrid progenies. Furthermore, the use of a wide genetic base tester made up of a bulk of pollen of several male parents was effective, since all of them were represented in progeny.

Both techniques allowed generating hybrid progenies of *P. notatum* improved for forage yield during the first selection and crossing cycle. However, the comparative evaluation between progenies obtained by RPS and RSCA shows that RPS produced a greater or equal progress compared with RSCA. The use of both methods allowed obtaining superior families for forage yield where dominance genetic effects predominated. This means that by RPS and RSCA favorable alleles with partial or complete dominance for forage yield were accumulated. Nonetheless, the use of RPS would be more suitable for improving *P. notatum*, since the progress for forage yield achieved by RPS was greater than or equal to that achieved by RSCA, and RPS is also easier, cheaper, and faster than RSCA.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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